

Procyanidin Content of Grape Seed and Pomace, and Total Anthocyanin Content of Grape Pomace as Affected by Extrusion Processing

R.C. KHANAL, L.R. HOWARD, AND R.L. PRIOR

ABSTRACT: Grape juice processing by-products, grape seed and pomace are a rich source of procyanidins, compounds that may afford protection against chronic disease. This study was undertaken to identify optimal extrusion conditions to enhance the contents of monomers and dimers at the expense of large molecular weight procyanidin oligomers and polymers in grape seed and pomace. Extrusion variables, temperature (160, 170, and 180 °C in grape seed, and 160, 170, 180, and 190 °C in pomace) and screw speed (100, 150, and 200 rpm in both) were tested using mixtures of grape seed as well as pomace with decorticated white sorghum flour at a ratio of 30:70 and moisture content of 45%. Samples of grape seed and pomace were analyzed for procyanidin composition before and after extrusion, and total anthocyanins were determined in pomace. Additionally, chromatograms from diol and normal phase high-performance liquid chromatography were compared for the separation of procyanidins. Extrusion of both grape by-products increased the biologically important monomer and dimers considerably across all temperature and screw speeds. Highest monomer content resulted when extruded at a temperature of 170 °C and screw speed of 200 rpm, which were 120% and 80% higher than the unextruded grape seed and pomace, respectively. Increases in monomer and dimer contents were apparently the result of reduced polymer contents, which declined by 27% to 54%, or enhanced extraction facilitated by disruption of the food matrix during extrusion. Extrusion processing reduced total anthocyanins in pomace by 18% to 53%.

Practical Applications: Extrusion processing can be used to increase procyanidin monomer and dimer contents in grape seed and pomace. Procyanidins in grape by-products have many health benefits, but most are present as large molecular weight compounds, which are poorly absorbed. Extrusion processing appears to be a promising technology to increase levels of the bioactive low molecular weight procyanidins.

Keywords: anthocyanins, catechin, epicatechin, extrusion, grape pomace, grape seed, LC/MS, procyanidins, tannins

Introduction

Procyanidins (PAs) or condensed tannins are the oligomeric and polymeric polyhydroxy flavan-3-ol units that are ubiquitous in plants and constitute the 2nd most abundant group of natural phenolics after lignin (Porter 1994). Their existence in common foods bears importance not only because they are partially responsible for organoleptic characteristics of foods like grapes, wines, and cocoa (Sarni-Manchado and others 1999), but also because of their reported antioxidant capacity and possible protective effects in human health (Facino and others 1999; Yamakoshi and others 1999; Zhu and others 1999; Broadhurst and others 2000; Santos-Buelga and Scalbert 2000; Wang and others 2000; Joshi and others 2001; Anderson and others 2004). Moreover, the oxygen radical absorbance capacity (ORAC) was found to correlate with the

procyanidin content in cocoa and chocolate (Adamson and others 1999).

Extrusion is one of the most common industrial processes used to make snacks, and its main goal is to enhance the overall digestibility and bioavailability of food nutrients. It simultaneously performs, among others, mixing, cutting, crushing, pressing, expanding, drying, and sterilizing functions (McDougall and others 1996). In particular, it provides shearing effects concomitantly at high temperature and pressure (Kokini 1993), thereby modifying the food matrix that cannot be acted upon by the digestive enzymes to release the associated nutrients. Since a substantial amount of procyanidins present in fruits, including grapes, is in a form not readily available for absorption, it is important to investigate how much of the procyanidins present in its seed and pomace is in monomeric and polymeric forms and whether extrusion brings about any change in the distribution of these polyphenols.

While monomeric and some oligomeric procyanidins have been found to be absorbed (Shoji and others 2006), polymeric procyanidins are poorly absorbed (Donovan and others 2002; Gonthier and others 2003). Processing techniques that can convert/degrade the

MS 20090188 Submitted 3/3/2009, Accepted 4/27/2009. Authors Khanal and Howard are with Dept. of Food Science, Univ. of Arkansas, Fayetteville, AR 72703, U.S.A. Author Prior is with USDA-ARS, Arkansas Children's Nutrition Center, Little Rock, AR 72202, U.S.A. Direct inquiries to author Howard (E-mail: lukeh@uark.edu).

higher molecular weight (MW) procyanidins may prove useful in increasing the monomers and other lower MW oligomers. A previous study demonstrated that extrusion of sorghum significantly enhanced lower MW procyanidins, but reduced higher MW procyanidins, including the polymers (Awika and others 2003). Our recent work on blueberry pomace demonstrated that monomers and lower-level oligomers could be substantially increased by extrusion (Khanal and others 2009). These studies led us to hypothesize that extrusion has the same potential to enhance the procyanidin monomer and dimer contents in grape seed and grape pomace, and improve the associated health benefits. In these experiments, extrusion variables, temperature, and screw speed, were studied to determine their effects on procyanidin composition of grape seed and grape pomace as well as total anthocyanin (ACY) content of grape pomace.

Materials and Methods

Sample preparation

Grape seed (variety Riesling) was obtained from FruitSmart (Prosser, Wash., U.S.A.), and grape pomace (variety Sunbelt) was obtained from the Univ. of Arkansas Enology program (Fayetteville, Ark., U.S.A.). The pomace consisting of stems, skins, and seeds was freeze dried before grinding in a coffee grinder. Grape seeds were screened through an nr 6 sieve to exclude any extraneous material and ground in a coffee grinder. Both materials were then mixed

with hammer milled decorticated white sorghum (Texas A&M Univ. Hybrid ATx436xRTx631 courtesy of Dr. L. Rooney, Cereal Quality Laboratory, Texas A&M Univ., College Station, Tex., U.S.A.) in a kitchen mixture (Kitchen Aid Classic Model K45SS, Benton Harbor, Mich., U.S.A.) at a ratio of 30 : 70 on a dry weight basis (a total of 2 kg/batch). Water was added to bring the moisture content to 45%. Speed of the kitchen mixture was set at 6. Reasons for using decorticated white sorghum and 45% moisture content have been described previously (Khanal and others 2009). All samples were stored at -20°C until further laboratory analyses.

Extrusion processing

A mixture of grape seed with decorticated white sorghum was extruded in 3 batches using a twin screw Haake PolyLab System extruder (Thermo Haake, Karlsruhe, Germany). Details about the extruder have been described previously (Khanal and others 2009). Extrusion was carried out at temperatures of 160, 170, 180, and 190°C with screw speeds of 100, 150, and 200 rpm. However, the temperature in zone A (at the mouth of the extruder) was set at 90°C as opposed to the other 3 zones. The temperature gradient minimized clogging of the extruder and enhanced the quality of extrudates. However, no data are reported for grape seed extruded at 190°C since it did not produce appreciable quality and quantity of extrudates at 100 and 150 rpm screw speeds. A continuous manual feeding (approximately 100 g/feeding) was employed during extrusion.

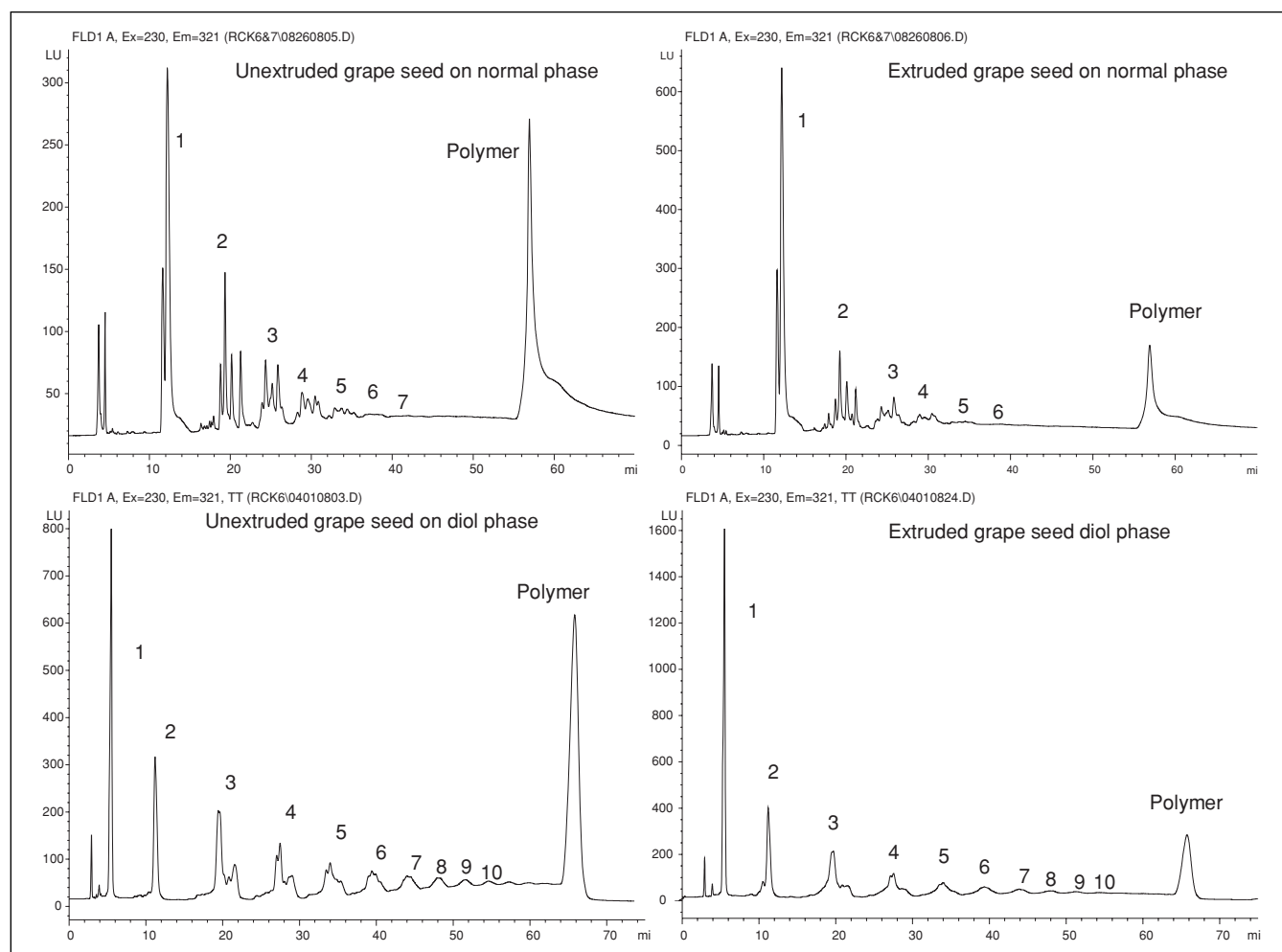


Figure 1 – Representative HPLC chromatograms of unextruded and extruded grape seed on normal and diol phases.

Extraction and analysis of procyanidins

Procyanidins were extracted and isolated as previously described (Khanal and others 2009) with a simple modification in which samples were soaked overnight in extraction solvent before extraction since it enhanced the extraction of procyanidins significantly ($P \leq 0.05$) in an experiment with 3 varieties of grape seeds (unpublished data). Moisture content of the samples was determined in a forced air oven at 103 °C for 8 h and procyanidin concentrations are expressed on dry weight (DW) basis.

Standards

Identification and quantification of the procyanidin peaks on the HPLC chromatogram was based on procyanidin standards prepared from purified cocoa (Gu and others 2003a) that contained monomers through decamers. A polymeric fraction (average DP of > 36.1 containing no procyanidins with DP < 10) purified from blueberry was used as polymer standard. Fractionation and characterization of both oligomer and polymer standards from cocoa and blueberries were described previously (Gu and others 2002).

High-performance liquid chromatography (HPLC) and HPLC-electrospray/ionization-mass spectrometry (HPLC-ESI-MS)

Procyanidins were analyzed using an Agilent 1100 HPLC system consisting of a quaternary pump, a solvent degasser, an autosampler, a thermostated column compartment, a diode-array detector, a fluorescence detector, and ChemStation for data collection and manipulation (Agilent Technologies, Palo Alto, Calif., U.S.A.). Ex-

tracts were passed through a 0.45- μ m filter. Separations based on degree of polymerization were conducted using a Develosil Diol column (250×4.6 , 5 μ m, Phenomenex, Torrance, Calif., U.S.A.) as described previously (Kelm and others 2006; Khanal and others 2009). A flow rate of 1 mL/min was maintained for a 75-min run. Mobile phase consisted of (A) 98:2 acetonitrile:acetic acid and (B) 95:3:2 methanol:water:acetic acid. The 75-min gradient was 0 to 3 min 7% B isocratic, 3 to 60 min 7% to 37.6% B linear, 60 to 63 min 37.6% to 100% linear, 63 to 70 min 100% B isocratic, 70 to 75 min 7% B linear followed by 10-min re-equilibration of the column. Procyanidin peaks were monitored by fluorescence detection with excitation at 230 nm and emission at 321 nm.

The HPLC was connected to a Bruker Esquire-LC ion trap mass spectrometer (Bruker Daltonics, Billerica, Mass., U.S.A.) to verify the procyanidin peaks with their molecular weights. After studying the HPLC chromatograms from both normal and diol phase separations of both grape seed and grape pomace, it was felt that quantitation of individual procyanidins was probably better with diol phase while identification of the linkages present within the individual procyanidins was better with the normal phase. As a result, data obtained from mass spectrometer were those from normal phase separation, which was carried out using a 5- μ m Luna silica column (250×4.6 mm) (Phenomenex) at a column temperature of 37 °C. The mobile phase consisted of (A) 82:14:2:2 methylene chloride:methanol:acetic acid:water, (B) 96:2:2 methanol:acetic acid:water. The 70-min gradient was 0 to 20 min 0% to 11.7% B linear; 20 to 50 min 11.7% to 25.6% B linear; 50 to 55 min 25.6% to 87.7% B linear, 55 to 65 min 87.7% B isocratic, and 65 to

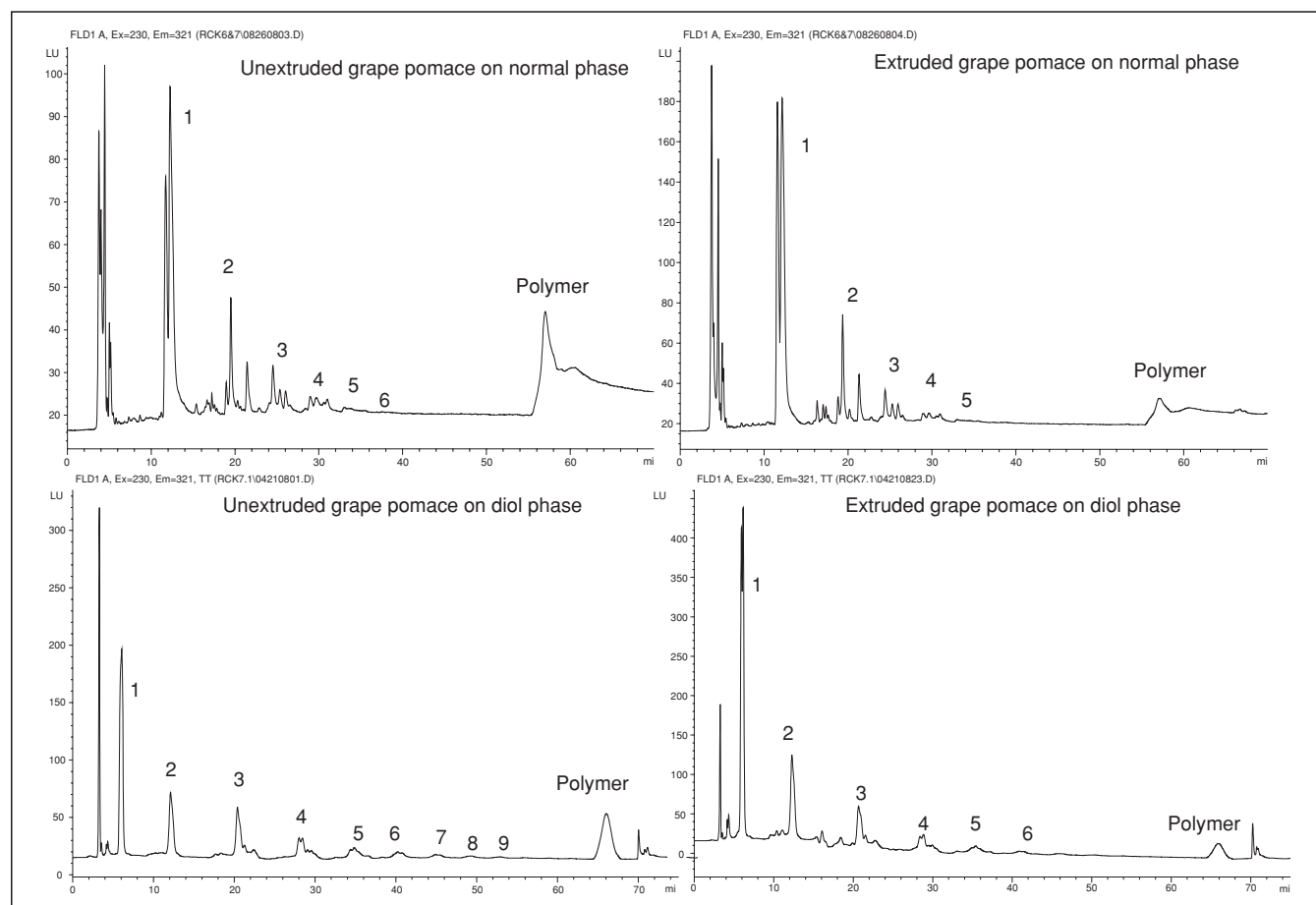


Figure 2—Representative HPLC chromatograms of unextruded and extruded grape pomace on normal and diol phases.

70 min 87.7% to 0% B linear followed by 10 min of re-equilibration of the column before the next run. Flow rate was maintained at 0.8 mL/min. For fluorescence detection, excitation and emission wavelengths were 230 and 321 nm. UV detection was carried out at 280 nm compared with a reference wavelength at 650 nm.

The eluting stream (1 mL/min) from the HPLC apparatus was introduced into the mass spectrometer. Ionization of procyanidins was enhanced using post-column 10 mmol/L ammonium acetate in methanol (Prior and others 2001). A flow rate of 0.06 mL/min was used for ammonium acetate, which was added via a tee in the eluant stream of the HPLC apparatus just prior to the mass spectrometer by an auxiliary HP 1100 series HPLC pump. The nitrogen pressure and flow rate on the nebulizer were 50 psi and 10 L/min, respectively, with a drying gas temperature of 350 °C. The capillary voltage was 3.5 kV. The scan range was set at m/z 150 to 2200. Details about the operational conditions of the mass spectrometer have been described previously (Khanal and others 2009).

Determination of total anthocyanins in grape pomace

Total anthocyanins were determined using the pH differential method (Giusti and Wrolstad 2001). Cyanidin 3-glucoside (c3g)

was used as the standard. Results were expressed as mg of c3g equivalents per kg DW.

Statistical analysis

Statistical analysis was carried out in JMP (SAS Inst. Inc., Cary, N.C., U.S.A.) in a 3-step process in both the experiments. First, the lowest increase in monomer contents was compared with unextruded control using a *t*-test. When a significant difference ($P \leq 0.05$) was observed (using monomer – the primary procyanidin that can be absorbed – as the determining constituent), temperature, screw speed, and their interaction were included as fixed factors in a factorial arrangement of 4×3 and 3×3 for grape pomace and grape seed, respectively, with 3 replicates in each experiment. It did not include the data obtained for unextruded control. When interaction was observed in major oligomers and polymer, combination of temperature and screw speed was used as the only fixed factor in a completely randomized model that also included control data. Such a stepwise process was necessary to determine the best extrusion conditions. Total ACY were analyzed using the same approach. Means were separated using least significant difference (LSD) test with significance declared at $P \leq 0.05$.

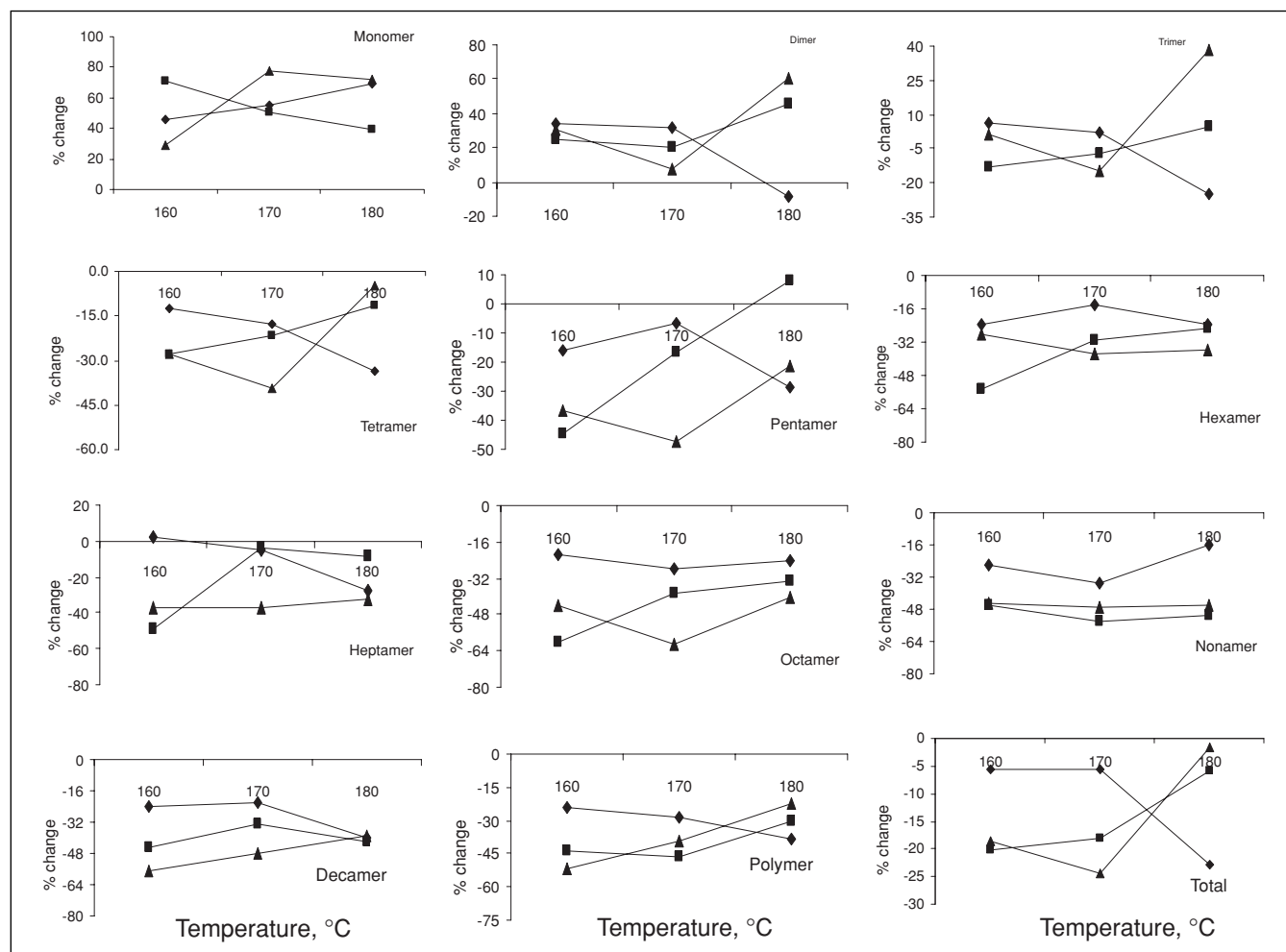


Figure 3—Effect of temperature and screw speed during extrusion on procyanidin composition (% change over control) of grape seed. Three levels each of temperature at 160, 170, and 180 °C and screw speed of 100 (◆), 150 (■), and 200 (▲) rpm were used for extruding grape seed in a PolyHaake Extruder.

Results and Discussion

Analysis of grape seed and grape pomace procyanidins

Retention times of external standards as well as the mass spectra obtained from HPLC-ESI-MS analyses were used to identify the procyanidin monomers through decamers and polymers. However, no mass spectral data are provided beyond heptamers. Fluorescence response curves obtained from both normal and diol phase HPLC separations for unextruded and extruded grape seed and grape pomace are presented in Figure 1 and 2, respectively. As can be observed, the fluorescence peak from normal phase separation demonstrated the presence of 2 monomers and 3 or more trimers, tetramers, and pentamers. Compound mass spectra (data not shown) confirmed that these oligomers consisted of (epi)catechin units that had exclusively B-type linkages. The presence or absence of A- or B-type linkages in hexamers and above was not determined. Since the 2 monomers co-eluted, we could not identify which one was (+)-catechin and which one was (–)-epicatechin. Although peak height in the chromatogram alone cannot determine the quantitative increase (or decrease) in individual procyanidins, an obvious difference in the peak size for some of the components, such as monomers, dimers, and polymers could be observed between extruded and unextruded products for both grape seed and grape pomace. This was apparent in both normal and diol phase chromatograms. No attempt was made to determine the DP of polymers. Although it may not be clear in the HPLC chromatogram, compound mass spectra confirmed the presence of 2 hexamers in both grape seed and grape pomace.

Closer examination of the chromatograms obtained from normal and diol phase separations suggested a rather interesting result about which method to employ during HPLC separation. While peaks obtained from diol phase were more pronounced and clean, normal phase separated compounds within the individual procyanidins better. Normal phase did not provide as clear of peaks for pentamers through decamers as did the diol phase. These data indicated that quantification would probably be carried out better by diol than normal phase. However, identification of the linkages and determination of the number and type of compounds within individual procyanidins would be carried out better by normal phase separation. We are currently comparing the normal and diol phase methods for the quantification of procyanidins in a number of food products. Regardless of the separation method used no new peaks were observed in extruded products of either grape seed or pomace.

One of our objectives was to study if extrusion affects the changes in the linkages present in procyanidin oligomers from grape seed or grape pomace. All of the oligomers we investigated had exclusively B-type linkages. This is in agreement with previous findings suggesting most of the foods, including grapes, contain exclusively B-type procyanidins (Gu and others 2003b). Extrusion had no effect on the linkages present in the procyanidins as could be observed with either diol phase (data not shown) or normal phase separations.

Procyanidins in grape and wine have been studied in-depth. Grape seeds contain partly galloylated procyanidins with catechin, epicatechin, and epicatechin gallate at a ratio of 1:3:1 being subunits (Priour and others 1994). Using MALDI-TOF mass spectrometry, Krueger and others (2000) detected procyanidin dimers through undecamers in grape seed with 0 to 7 gallic acid substitutions. The DP of up to 28 has been tentatively determined in a grape seed

Table 1 – Effect of extrusion temperature and screw speed on procyanidin^a composition (mg/kg DW, mean \pm SEM) of grape seed.

| Treatment ^b | DP1 | DP2 | DP3 | DP4 | DP5 | DP6 | DP7 | DP8 | DP9 | DP10 | Polymer | Total |
|------------------------|------------------------------|------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|----------------------------|-----------------------------|-------------------------------|-----------------------------|------------------------------|-------------------------------|
| Control | 4228 \pm 187 ^{cd} | 1369 \pm 76 ^c | 1964 \pm 156 ^b | 1977 \pm 203 ^a | 1653 \pm 128 ^{ab} | 2008 \pm 120 ^a | 802 \pm 75 ⁿ | 1160 \pm 128 ^a | 1982 \pm 171 ^a | 714 \pm 95 ^a | 8590 \pm 625 ^a | 26446 \pm 1808 ^a |
| 160/100 | 6172 \pm 240 ^{cd} | 1830 \pm 103 ^{bc} | 2091 \pm 179 ^b | 1729 \pm 89 ^{abc} | 1393 \pm 75 ^{bcd} | 1538 \pm 72 ^{bcd} | 817 \pm 14 ^a | 912 \pm 73 ^{ab} | 1461 \pm 135 ^{bc} | 542 \pm 40 ^{abc} | 6490 \pm 309 ^b | 24974 \pm 808 ^a |
| 160/150 | 7223 \pm 310 ^{ab} | 1805 \pm 73 ^{bc} | 2013 \pm 105 ^b | 1622 \pm 3 ^{bcd} | 1544 \pm 61 ^{abc} | 1721 \pm 128 ^b | 759 \pm 32 ^a | 837 \pm 53 ^b | 1279 \pm 286 ^{bcd} | 560 \pm 99 ^{ab} | 6170 \pm 526 ^{bc} | 25533 \pm 689 ^a |
| 160/200 | 5460 \pm 145 ^d | 1252 \pm 62 ^e | 1476 \pm 61 ^c | 1309 \pm 42 ^{ef} | 1175 \pm 161 ^{def} | 1543 \pm 28 ^{bc} | 580 \pm 77 ^{bc} | 883 \pm 120 ^{ab} | 1659 \pm 13 ^{ab} | 431 \pm 50 ^{bcd} | 5325 \pm 169 ^{cd} | 21095 \pm 568 ^b |
| 170/100 | 6554 \pm 280 ^{bc} | 1707 \pm 125 ^{cd} | 1701 \pm 100 ^{bc} | 1430 \pm 17 ^{def} | 914 \pm 47 ^{fg} | 917 \pm 78 ^f | 408 \pm 52 ^c | 461 \pm 7 ^c | 1076 \pm 107 ^{cd} | 396 \pm 49 ^{bcd} | 4844 \pm 296 ^{de} | 20407 \pm 604 ^b |
| 170/150 | 6377 \pm 245 ^c | 1646 \pm 60 ^{cd} | 1829 \pm 205 ^{bc} | 1551 \pm 152 ^{cde} | 1381 \pm 99 ^{bcd} | 1382 \pm 104 ^{cde} | 769 \pm 81 ^a | 711 \pm 120 ^{bc} | 915 \pm 100 ^d | 478 \pm 35 ^{bcd} | 4601 \pm 276 ^{de} | 21640 \pm 813 ^b |
| 170/200 | 7508 \pm 236 ^a | 1996 \pm 77 ^{ab} | 2049 \pm 174 ^b | 1753 \pm 60 ^{abc} | 1788 \pm 42 ^a | 1501 \pm 132 ^{bcd} | 736 \pm 54 ^{ab} | 775 \pm 59 ^b | 967 \pm 149 ^d | 413 \pm 74 ^{bcd} | 5964 \pm 203 ^{bc} | 25447 \pm 414 ^a |
| 180/100 | 7152 \pm 84 ^{ab} | 1787 \pm 68 ^{bc} | 1986 \pm 67 ^b | 1431 \pm 89 ^{def} | 1047 \pm 40 ^{efg} | 1432 \pm 38 ^{cde} | 500 \pm 83 ^c | 653 \pm 191 ^{bc} | 1092 \pm 79 ^{cd} | 305 \pm 33 ^d | 4147 \pm 278 ^e | 21532 \pm 350 ^b |
| 180/150 | 5901 \pm 268 ^{cd} | 1473 \pm 95 ^{de} | 1675 \pm 152 ^{bc} | 1198 \pm 39 ^f | 870 \pm 92 ^g | 1246 \pm 57 ^e | 502 \pm 69 ^c | 455 \pm 99 ^c | 1057 \pm 148 ^{cd} | 372 \pm 43 ^d | 5248 \pm 444 ^{cd} | 19995 \pm 156 ^b |
| 180/200 | 7271 \pm 428 ^{ab} | 2204 \pm 74 ^a | 2718 \pm 211 ^a | 1886 \pm 39 ^{ab} | 1300 \pm 122 ^{cde} | 1287 \pm 31 ^{de} | 546 \pm 14 ^c | 693 \pm 16 ^{bc} | 1071 \pm 98 ^{cd} | 437 \pm 56 ^{bcd} | 6646 \pm 177 ^b | 26058 \pm 861 ^a |

^aProcyanidin composition is presented as degree of polymerization (DP), in which DP1 means monomer, DP2 dimer, and so on. All procyanidins > DP10 are grouped as one and described in the text as polymer.

^bFirst part of the treatment represents temperature in °C and the 2nd part screw speed in rpm.

^cFigures in the same column with different superscripts are statistically different ($P \leq 0.05$).

procyanidin extract using ESI MS with the degree of galloylation up to eight (Hayasaka and others 2003). Procyanidins in grape skin contain catechin, epicatechin, epigallocatechin, and epicatechin-3-*O*-gallate as constitutive units (Souquet and others 1996). In comparison to procyanidins in grape seed, grape skins have a higher degree of polymerization and lower proportion of galloylated subunits (Gu and others 2003b). We did not attempt to determine the degree of polymerization of procyanidins in either grape seed or pomace nor did we attempt to identify their constituent units in the current study. Since procyanidins extracted from wine have similar composition as that of grape skins (Monagas and others 2003), and pomace contains skins, pulp, and some seeds, grape pomace may contain the constituent units that are contributed by both the skins and the seeds.

Procyanidin composition and content of grape seed and grape pomace

Grape seed had considerable amounts of total procyanidins, including the monomers (Table 1). The total procyanidin content in the unextruded grape seed was 2.64% on DW basis; of which 16% was the monomer (4228 mg/kg DW) and approximately 33% the polymer (8590 mg/kg). Dimers and trimers, which have been shown to be absorbed in the intestine (Baba and others 2002; Holt and others 2002; Tsang and others 2005) contributed 5% and 7% of the DW, respectively. On the other hand, grape pomace had considerably lower total procyanidin contents (Table 2) than grape seed, which was only 16% (or 0.43% of the DW) of the amount present in grape seed. Although monomer content (1609 mg/kg DW) in grape pomace was proportionately higher than in grape seed, it was only 38% of the amount present in grape seed. Similarly, dimer and trimer contents in grape pomace were also much lower compared to grape seed. Proportionately though, grape pomace had higher levels of lower-level oligomers than grape seed. Conversely, polymer content (905 mg/kg DW) of grape pomace was proportionately (21%) lower compared to grape seed.

Effect of extrusion on procyanidin composition and content

Extrusion enhanced ($P \leq 0.05$) the biologically important monomer as well as dimer contents by as much as 80% over the unextruded control in grape seed. The best results were obtained when extruded at 170 °C and 200 rpm for monomer, and 180 °C and 200 rpm for dimer contents. A representative chromatogram (Figure 1) of the extruded and unextruded grape seed clearly

demonstrates the substantial increase in monomer and dimer content, probably by the conversion of some higher-level oligomers and polymers into their lower oligomer counterparts. Trimer contents were either reduced or not affected by extrusion, except at 180 °C and 200 rpm which resulted in a significant increase ($P \leq 0.05$). All other oligomers were reduced ($P \leq 0.05$) under most of the extrusion conditions. A significant reduction in polymer contents occurred across all temperatures and screw speeds with the highest decrease occurring at 190 °C and 100 rpm, a condition that did not correspond exactly with the highest increase in monomer contents.

Extrusion enhanced monomer, dimer, and trimer contents of grape pomace across all temperature and screw speeds by as much as 120% in the case of monomer at 170 °C and 200 rpm (Table 2), which is the same condition that resulted in the highest increase in monomer contents with grape seed. Transition occurred at the level of tetramer when certain extrusion conditions would enhance while others would reduce it. Similar to grape seed, highest decrease in polymer contents of extruded grape pomace occurred at 190 °C and 100 rpm, a condition that did not correspond exactly with the highest increase in monomer contents. While pentamers, hexamers, and polymers were reduced significantly ($P \leq 0.05$) across all extrusion conditions, heptamers through decamers were either not detected or present in very small amounts in the extruded products even though they were present in unextruded pomace and therefore, not reported here.

Since interaction between temperature and screw speed during extrusion was observed with grape seed as well as grape pomace, their effects are presented in a series of graphs in Figure 3 (grape seed) and Figure 4 (grape pomace). With grape seed, monomer contents increased when extrusion temperature was increased from 160 to 170 °C, but no further increase was observed with increasing temperature. Increasing the residence time inside the extruder barrel at 160 °C enhanced grape seed monomer content linearly, but doing so at higher temperatures of 180 and 190 °C did not produce the same results. Although dimer contents were primarily increased at all temperature and screw speeds, effect of temperature and screw speed was variable for the trimer. Extrusion of grape seed at 190 °C was rather difficult with the extruder we had and did not produce quality extrudates at either 100 or 150, but only at 200 rpm of screw speed. Even though extrusion at 200 rpm produced quality extrudates, no data are presented here because the increase in monomer contents was significantly ($P \leq 0.05$) smaller compared to 170 °C and 200 rpm or some other extrusion conditions.

Table 2 – Effect of extrusion temperature and screw speed on procyanidin^A composition (mg/kg DW, mean \pm SEM) of grape pomace.

| Treatment ^B | DP1 | DP2 | DP3 | DP4 | DP5 | DP6 | Polymer | Total |
|------------------------|--------------------------------|-----------------------------|-----------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------|-------------------------------|
| Control | 1609 \pm 87 ^{hC} | 329 \pm 24 ^e | 384 \pm 34 ^d | 348 \pm 23 ^{ab} | 364 \pm 40 ^a | 365 \pm 5 ^a | 905 \pm 34 ^a | 4304 \pm 148 ^e |
| 160/100 | 2839 \pm 111 ^{defg} | 504 \pm 23 ^d | 395 \pm 4 ^d | 331 \pm 21 ^{ab} | 263 \pm 4 ^{bcd} | 348 \pm 43 ^{ab} | 577 \pm 18 ^{bc} | 5256 \pm 70 ^d |
| 160/150 | 2691 \pm 108 ^{fg} | 538 \pm 18 ^d | 499 \pm 26 ^c | 379 \pm 57 ^{ab} | 256 \pm 47 ^{bcd} | 280 \pm 25 ^{bc} | 471 \pm 50 ^d | 5115 \pm 208 ^d |
| 160/200 | 2752 \pm 64 ^{efg} | 581 \pm 30 ^{cd} | 548 \pm 12 ^{abc} | 305 \pm 8 ^{bc} | 209 \pm 16 ^{cde} | 285 \pm 16 ^{bc} | 505 \pm 15 ^d | 5184 \pm 126 ^d |
| 170/100 | 3269 \pm 96 ^b | 732 \pm 35 ^a | 593 \pm 33 ^{ab} | 346 \pm 20 ^{ab} | 266 \pm 28 ^{bc} | 213 \pm 23 ^{cd} | 495 \pm 21 ^d | 5914 \pm 185 ^b |
| 170/150 | 2880 \pm 99 ^{cdef} | 647 \pm 61 ^{abc} | 581 \pm 68 ^{abc} | 328 \pm 46 ^{ab} | 241 \pm 27 ^{bcd} | 220 \pm 39 ^{cd} | 503 \pm 17 ^d | 5401 \pm 314 ^{cd} |
| 170/200 | 3587 \pm 33 ^a | 724 \pm 30 ^{ab} | 629 \pm 30 ^a | 404 \pm 22 ^a | 303 \pm 11 ^{ab} | 274 \pm 18 ^{bc} | 531 \pm 18 ^{bcd} | 6453 \pm 150 ^a |
| 180/100 | 2981 \pm 142 ^{cde} | 634 \pm 20 ^{bc} | 530 \pm 14 ^{bc} | 361 \pm 38 ^{ab} | 242 \pm 16 ^{bcd} | 221 \pm 4 ^{cd} | 500 \pm 26 ^d | 5468 \pm 23 ^{bcd} |
| 180/150 | 3092 \pm 160 ^{bcd} | 688 \pm 33 ^{ab} | 535 \pm 14 ^{bc} | 342 \pm 15 ^{ab} | 190 \pm 25 ^{de} | 256 \pm 12 ^c | 503 \pm 25 ^d | 5605 \pm 165 ^{bcd} |
| 180/200 | 3278 \pm 30 ^b | 673 \pm 11 ^{ab} | 566 \pm 21 ^{abc} | 363 \pm 13 ^{ab} | 238 \pm 12 ^{bcd} | 258 \pm 44 ^c | 473 \pm 5 ^d | 5850 \pm 237 ^{bc} |
| 190/100 | 2590 \pm 69 ^g | 524 \pm 6 ^d | 398 \pm 24 ^d | 233 \pm 16 ^c | 170 \pm 28 ^e | 153 \pm 19 ^d | 370 \pm 16 ^e | 4469 \pm 38 ^e |
| 190/150 | 3092 \pm 127 ^{bcd} | 653 \pm 37 ^{abc} | 516 \pm 28 ^{bc} | 328 \pm 34 ^{ab} | 224 \pm 22 ^{cde} | 226 \pm 3 ^{cd} | 517 \pm 30 ^{cd} | 5556 \pm 165 ^{bcd} |
| 190/200 | 3144 \pm 42 ^{bc} | 736 \pm 40 ^a | 570 \pm 13 ^{abc} | 342 \pm 30 ^{ab} | 238 \pm 19 ^{bcd} | 172 \pm 41 ^d | 589 \pm 6 ^b | 5791 \pm 173 ^{bc} |

^AProcyanidin composition is presented as degree of polymerization (DP), in which DP1 means monomer, DP2 dimer, and so on. All procyanidins > DP10 are grouped as one and described in the text as polymer. Oligomers DP7 through DP10 were not detected very well in many cases and therefore not presented.

^BFirst part of the treatment represents temperature in °C and the 2nd part screw speed in rpm.

^CFigures in the same column with different superscripts are statistically different ($P < 0.05$).

As stated previously, monomers, dimers, trimers, and even tetramers increased the most at 170 °C and 200 rpm for grape pomace, which was true only for the monomer in the case of grape seed. While monomers through tetramers increased linearly from 160 to 170 °C with increasing residence time inside the extruder barrel, their enhancement declined linearly with the increasing residence time at higher temperatures of 180 and 190 °C, which was similar to grape seed. Similarly, enhancement in total procyanidin content in grape pomace followed similar pattern as monomers.

One striking difference about the effect of extrusion between grape seed and grape pomace was observed on total procyanidin contents of the 2 materials. While extrusion enhanced the total

procyanidin contents significantly ($P \leq 0.05$) in grape pomace (as much as 49%), it was either reduced or remained similar in grape seed. It substantiates one of the propositions we made previously (Khanal and others 2009) that part of the increase in monomer and lower-level oligomer contents may have come from enhanced extraction facilitated by disruption of the pomace matrix due to shearing effects concomitantly at high temperature and pressure (Kokini 1993). Since pomace contains pulp as well as some stems and leaves (in addition to some seeds) that have more fiber than seed, and extrusion provides shearing effect on the material, it is possible that it may actually help release more procyanidins during extraction from pomace than seed.

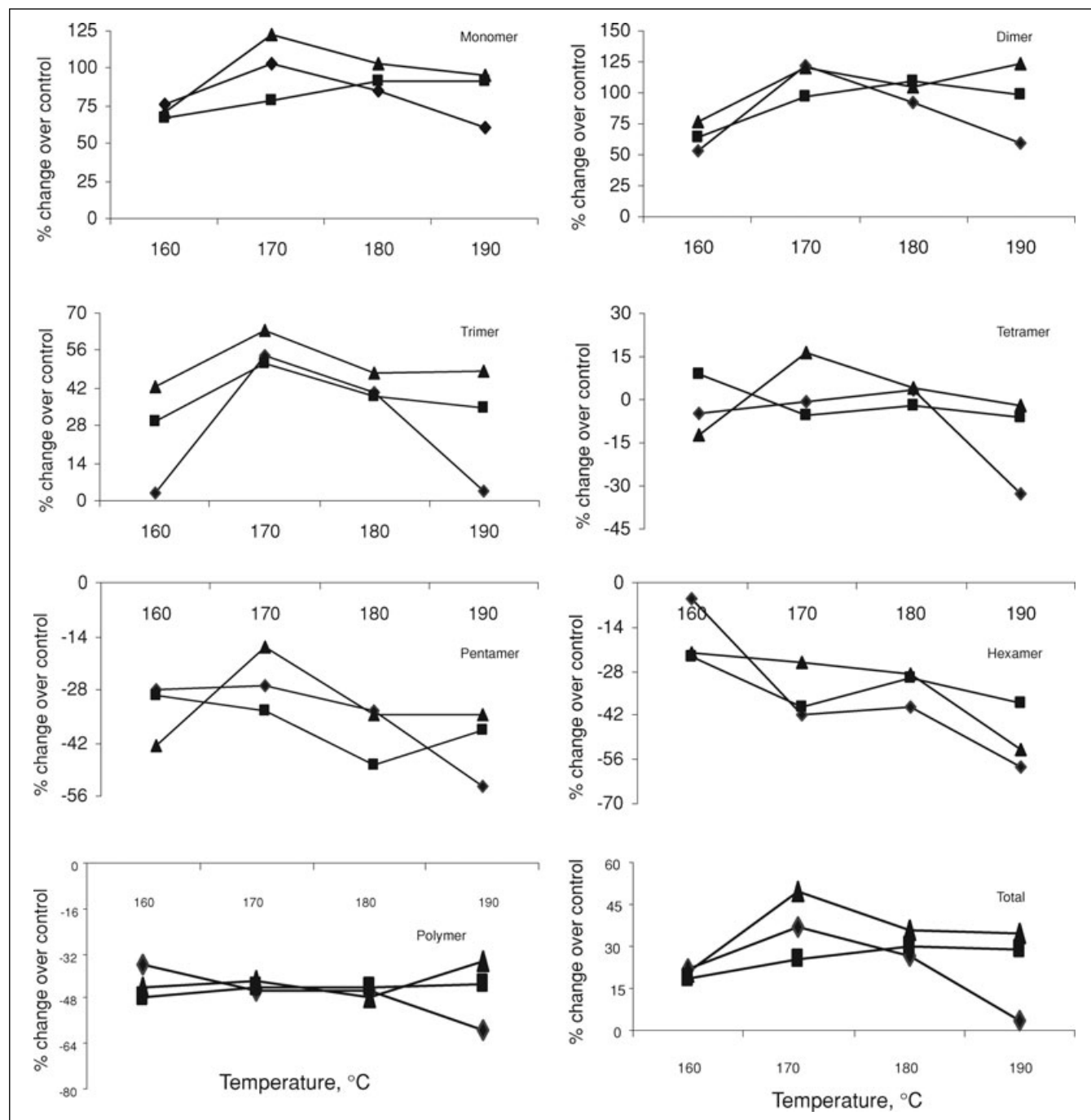


Figure 4—Effect of temperature and screw speed during extrusion on procyanidin composition (% change over control) of grape pomace. Four levels of temperature at 160, 170, 180, and 190 °C and 3 levels of screw speed at 100 (◆), 150 (■), and 200 (▲) rpm were used for extruding grape pomace in a PolyHaake Extruder.

We have recently reported that extrusion significantly ($P \leq 0.05$) increased monomer, dimer, and trimer contents in blueberry pomace and concomitantly decreased the polymer contents (Khanal and others 2009). Although Haake is a small pilot scale extruder, its results could be expected to be reproduced with a larger extruder under similar extrusion conditions. Indeed, extrusion enhanced monomer through tetramers by up to 65% in high tannin sorghum in one experiment (Gu and others 2008) and more than 100 to 400% of extractable monomers, dimers, and trimers in the other (Awika and others 2003) with a concomitant decrease in higher-level oligomers and polymers in both. We have also observed a 15% to 40% increase in monomer contents with both blueberry and grape pomace using a single screw extruder (data not reported) even at lower temperatures (150 °C). Both the increase in monomer contents and decrease in polymer contents in the current study were higher compared to previous studies in high tannin sorghum and blueberry pomace (Gu and others 2008; Khanal and others 2009). These studies suggest that the extent of increase in the lower MW procyanidins by extrusion processing may be dependent on the food matrix and extruder type.

We have proposed 3 possibilities for the enhanced monomer and lower-level oligomer contents previously (Khanal and others 2009). In light of the increased total contents of procyanidins, at least in the case of grape pomace, and reduction in the higher-level oligomers and polymers across all the extrusion conditions, it is possible that conversion as well as enhanced extraction may have actually contributed to the increased monomers, dimers, and some other smaller oligomers. While a certain amount of the polymer may have been converted to monomer and lower oligomers, not all polymer lost during extrusion was accounted for by the increase in monomer and lower-level oligomers. Moreover, studies with apple pomace have demonstrated that large MW procyanidins may bind irreversibly to cell-wall polysaccharides through hydrogen bonding and/or hydrophobic interactions (Renard and others 2001; Le Bourvellec and others 2004) and therefore, not all polymers and higher-level oligomers lost during extrusion may get converted into monomers and lower-level oligomers. Unavailability of sufficient amounts of purified polymers or higher-level oligomers for extrusion studies has made it difficult to determine exactly how much of the increase may have been contributed by enhanced extraction and how much through conversion.

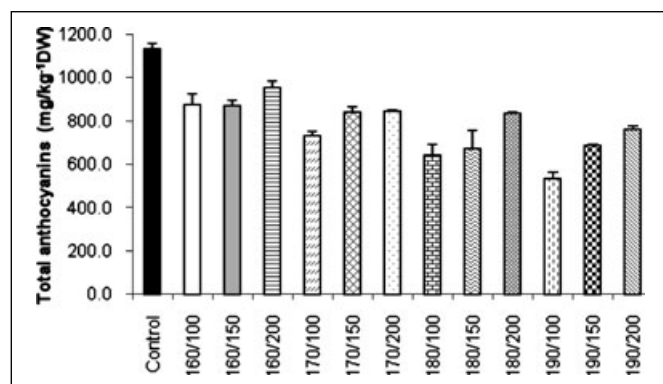


Figure 5—Total anthocyanin contents (mg/kg DW) in grape pomace before and after extrusion at various temperature and screw speeds. The 1st part of the treatment represents temperature in °C and the 2nd part represents screw speed in rpm.

Effect of extrusion on total anthocyanin content of grape pomace

Extrusion of grape pomace reduced the total ACY content ($P \leq 0.05$) and the reduction occurred across all extrusion conditions (Figure 5). Whereas the total ACY content of unextruded pomace was 1134 mg/kg DW, it was reduced to 530 mg/kg DW at the highest temperature and lowest screw speed. Both the values were lower than the values reported earlier for whole grapes (Wu and others 2006), which was expected as grape ACY are degraded during juice mashing steps and appreciable amounts are expressed into the juice (Auw and others 1996; Fuleki and Ricardo-Da-Silva 2003). Both temperature and screw speed, but not their interaction, affected total ACY contents ($P \leq 0.05$). While increasing extrusion temperature decreased the total ACY content linearly, increasing the screw speed minimized the extent of such reduction linearly. Increasing the screw speed from 100 to 200 rpm reduced the residence time of the material inside the extruder barrel, thus minimizing the exposure to high temperature that is responsible for ACY loss. The reduction in total ACY content (18% to 53%) was lower than previously observed (between 64% and 90%) with blueberry concentrates during extrusion (Camire and others 2002; Chaovanalikit and others 2003; Camire and others 2007). We observed 33% to 42% total ACY loss previously when blueberry pomace was extruded under similar conditions using the same extruder and at the same feed moisture contents (Khanal and others 2009).

Conclusions

Extrusion appears to be equally effective for enhancing the biologically important monomers and lower-level oligomer contents in grape seed and grape pomace as has been observed previously in blueberry pomace. Extrusion at 170 °C and 200 rpm resulted in the highest increase in monomer contents with 120% increase in grape pomace and 80% increase in grape seed over the unextruded control. All the lower-level procyanidins were exclusively of B-type. Although most extrusion conditions can significantly reduce total anthocyanin contents, the optimal temperature and screw speed that resulted in highest concentrations of monomers and lower-level procyanidin oligomers resulted in greater retention of anthocyanins than previous studies. It is very important to determine whether and how much of such increases in the monomers and dimers may have been due to conversion or enhanced extraction and whether such increases in monomer and dimer contents could be translated into perceived health benefits *in vivo*. The extrudates have a bland flavor and will require additional formulation work followed by sensory evaluation to determine consumer acceptability of the products.

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